

Claims

1. A method for transfecting T cells with a nucleic acid molecule comprising a gene, such that a gene is expressed in the T cells, comprising:

5 contacting T cells with at least one stimulatory agent, wherein the T cells are proliferating prior to contact with the at least one stimulatory agent, forming stimulated proliferating T cells; and

introducing a nucleic acid molecule comprising a gene into the proliferating, stimulated T cells such that the gene is expressed in the T cells.

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2. The method of claim 1, wherein the T cells are contacted with at least one proliferative agent which stimulates proliferation of the T cells prior to being contacted with the at least one stimulatory agent.

15 3. The method of claim 1, wherein the T cells are primary T cells.

4. The method of claim 1, wherein the at least one stimulatory agent is a combination of a first agent which provides a primary activation signal to the T cells and a second agent which provides a costimulatory signal to the T cells.

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5. The method of claim 4, wherein the first agent interacts with the T cell receptor/CD3 complex.

25 6. The method of claim 5, wherein the first agent is an anti-CD3 antibody.

7. The method of claim 4, wherein the first agent interacts with a CD2 molecule on the T cells.

30 8. The method of claim 4, wherein the first agent is an antigen on an antigen presenting cell.

9. The method of claim 4, wherein the second agent is an anti-CD28 antibody.

35 10. The method of claim 6, wherein the second agent is a stimulatory form of a natural ligand of CD28.

11. The method of claim 10, wherein the stimulatory form of a natural ligand of CD28 is the B lymphocyte antigen B7-1

12. The method of claim 10, wherein the stimulatory form of a natural ligand of CD28 is the B lymphocyte antigen B7-2.

13. The method of claim 1, wherein the at least one stimulatory agent is a combination of 5 a phorbol ester and a calcium ionophore.

14. The method of claim 1, wherein at least one stimulatory agent comprises a protein tyrosine kinase activator.

10 15. The method of claim 1, wherein at least one stimulatory agent is a super-antigen.

16. The method of claim 1, wherein the T cells are contacted with at least one stimulatory agent at most about 24 hours before introducing the nucleic acid molecule into the T cells.

15 17. The method of claim 16, wherein the cells are contacted with the stimulatory agent between about 1 and 24 hours before introducing the nucleic acid molecule into the T cells.

18. The method of claim 17, wherein the cells are contacted with the stimulatory agent about 10 hours before introducing the nucleic acid molecule into the T cells.

20 19. A method for transfecting primary T cells of a subject with a nucleic acid molecule comprising a gene such that the gene is expressed in the T cells, comprising obtaining T cells from the subject; contacting the T cells with at least one proliferative agent which stimulates proliferation of the T cells, forming proliferating T cells;

25 contacting the proliferating T cells with at least one stimulating agent, forming stimulated proliferating T cells; introducing the nucleic acid molecule comprising a gene into the stimulated proliferating T cells such that the gene is expressed in the T cells.

30 20. The method of claim 19 further comprising readministrating the T cells to the subject.

21. The method of claim 20, wherein the T cells are further stimulated *in vitro* to increase 35 the number of T cells prior to readministration to the subject.